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New insights into liver injury and regeneration from single-cell transcriptomics

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ABSTRACT

Recent advancements in single-cell transcriptomics have significantly enhanced our understanding of acute liver injury (ALI) and regeneration. These technologies enable high-resolution profiling of individual cells, uncovering the diverse and dynamic responses of liver cells to injury. By integrating single-cell RNA sequencing (scRNA-seq) with spatial transcriptomics, researchers have elucidated the complex cellular and molecular mechanisms driving ALI and regeneration processes. This review summarises the platforms and analytical methods of single-cell transcriptomics in liver research and discusses liver sample processing considerations for scRNA-seq, including the comparison between *in vivo* enzymatic perfusion and *ex vivo* digestion. It highlights the transformative impact of single-cell transcriptomics on ALI and regeneration studies, revealing novel insights into previously unrecognised cell subpopulations. Key discoveries include the identification of fetal-like and *Anxa2*$^{+}$ migratory hepatocytes at injury-repair interfaces, the characterisation of distinct activated hepatic stellate cell states during injury and repair phases, and the elucidation of zone-specific endothelial cell responses in *Wnt* signalling. Notable findings in macrophage heterogeneity include the discovery of *Trem2*$^{+}$ populations in both Kupffer cells and monocyte-derived macrophages, with distinct roles in injury and repair processes. The application of single-cell transcriptomics continues to hold promise for identifying novel therapeutic targets, ultimately advancing the development of novel treatment strategies for acute liver diseases.

INTRODUCTION

Acute liver injury (ALI) occurs when the liver experiences a variety of insults, leading to rapid hepatocyte death and inflammation.[1 2] Following ALI, the liver possesses an exceptional capacity for regeneration.[3] If the liver cannot adequately repair and regenerate itself, ALI may progress to acute liver failure (ALF), which carries a high risk of mortality or the need for liver transplantation.[1 2]

Traditionally, liver injury and regeneration studies have relied on whole-tissue analysis, obscuring the diversity and interplay of individual cell types. This limitation has been addressed by single-cell technologies, such as single-cell RNA sequencing (scRNA-seq), enabling high-resolution profiling of transcriptional activities at the single-cell level.[4] These approaches have uncovered previously unrecognised cell subpopulations, including rare and transient states, that play critical roles in liver pathology and repair (table 1).[4–25] Moreover, the emergence of spatial transcriptomics (ST) has revealed the zonal organisation of cell populations and their crosstalk within the liver microenvironment during processes of injury and regeneration (table 1).[4 5 11 12 15 16 25] Recent advances in single-cell transcriptomics have provided transformative insights into the heterogeneity and dynamics of liver cells during injury and regeneration and offered new perspectives on potential therapeutic targets. This review seeks to provide an overview of the application of single-cell transcriptomics in the research of ALI and regeneration. It also aims to highlight recent progress in understanding cellular and molecular mechanisms underlying ALI and regeneration through the use of single-cell technologies.

SINGLE-CELL TRANSCRIPTOMICS IN LIVER RESEARCH

ScRNA-seq is one of the most popular tools for studying cellular heterogeneity in fresh liver tissues, which requires immediate processing and enzymatic digestion. Alternatively, single-nucleus RNA sequencing (snRNA-seq) provides an advantageous approach by isolating nuclei from cells or tissues, allowing for greater flexibility when using either fresh or frozen liver samples, particularly in the context of human liver studies[26] (table 2). Moreover, snRNA-seq may be preferable for extremely small and/or easily degradable specimens.[26–28] A study showed that snRNA-seq could sufficiently measure transcripts from hepatocytes, cholangiocytes, mesenchymal cells (including fibroblasts and hepatic stellate cells (HSCs)),

Table 1 Studies of single-cell transcriptomics in ALI and repair

Study	Species/model	Platform	Lineages represented	Key findings	Single-cell isolation method	Data availability/ weblink	Reference
Pericentral injury/repair							
Umbaugh *et al* (2021)	Mouse APAP (injury—2 hours)	10x Chromium	Epithelia	Describe the heterogenous response of periportal and pericentral hepatocytes to APAP challenge.	*In vivo* enzymatic perfusion, hepatocytes isolated	N/A	7
Li *et al* (2023)	Mouse APAP (injury—3 hours)	Singleron SCOPE-chip	Epithelia Immune Endothelia	Describe NPC heterogeneity during early responses to APAP challenge.	Tissue Dissociation System (Singleron Biotech)	GEO GSE228305	8
Kolodziejczyk *et al* (2020)	Mouse APAP (injury—20 hours)	10x Chromium	Immune Endothelia Mesenchyme	Identify activated HSCs, ECs and KCs under MYC-dependent programme in driving ALI.	*In vivo* enzymatic perfusion, NPC merged with sorted HSCs	ArrayExpress E-MTAB-8263	9
Walesky *et al* (2020)	Mouse APAP	Seq-Well	Epithelia	Elucidate transcriptional shifts in hepatocytes at different zones during liver repair.	*In vivo* enzymatic perfusion	GEO GSE136679	10
Matchett *et al* (2024)	Human APAP/NAE-ALF Mouse APAP	10x Chromium (sn) Visium	Epithelia MPs Endothelia Mesenchyme	Identify *Anxa2*$^{+}$ migratory hepatocytes that mediate wound closure following ALI.	N/A	GEO GSE223561 https://liverregenerationatlas.hendersonlab.mvm.ed.ac.uk	11
Ben-Moshe *et al* (2022)	Mouse APAP (repair)	10x Chromium Visium	Epithelia Immune Endothelia Mesenchyme	Describe spatiotemporal dynamics of hepatocytes, HSCs and ECs during repair post-APAP; identify fetal-like hepatocytes; identify *Mmp12*$^{+}$ activated KCs.	*In vivo* enzymatic perfusion	Zenodo 6035873 https://itzkovitzapapp.weizmann.ac.il/apap/	12
Song *et al* (2023)	Mouse APAP (repair)	10x Chromium	Myeloid cells	Discover type 1 interferon and CSF1-mediated neutrophil-macrophage interaction in promoting liver repair after APAP-related ALI.	GentleMACS Tissue Dissociator (Miltenyi Biotec), CD11b+ cells selected by Microbead (Miltenyi Biotec)	N/A	13
Umbaugh *et al* (2024)	Mouse APAP (repair)	10x Chromium	Epithelia Immune Endothelia Mesenchyme	Identify p21^{+} hepatocytes surrounding the necrotic areas that impair liver recovery through CXCL14.	*In vivo* enzymatic perfusion	GEO GSE255835	14
De Ponti *et al* (2025)	Mouse APAP (repair)	10x Chromium Visium	Myeloid cells	Identify lipid-associated macrophage-like KCs and demonstrate their function in dying cell clearance and liver repair.	*In vivo* enzymatic perfusion, stain with CITE-seq antibodies	GEO GSE280514 GEO GSE280515 GEO GSE280652 https://www.livercellatlas.org/	15
Wang *et al* (2024)	Mouse CCl_4 (repair)	10x Chromium Visium	Epithelia	Reveal spatiotemporal dynamics of molecular pathology throughout liver lobules during injury and repair; discover hepatocyte-derived mTORC1 signalling in pericentral regeneration.	*In vivo* enzymatic perfusion, CYP2E1+ hepatocytes sorted	Genome Sequence Archive CRA007191	16
Zhao *et al* (2019)	Mouse CCl_4 (repair)	10x Chromium	Immune Endothelia Mesenchyme	Identify ECs being the major source of *Wnt2* and *Wnt9b* during repair after CCl_4 insult.	*In vivo* enzymatic perfusion, NPCs captured	N/A	17
Li *et al* (2023)	Mouse CCl_4 (repair—day 28)	10x Chromium	Monocytes/ macrophages	Describe the heterogeneity of KCs at the end of liver repair.	*In vivo* enzymatic perfusion	Genome Sequence Archive CRA007105	18
Post-transplantation injury							

Continued

Table 1 Continued

Study	Species/model	Platform	Lineages represented	Key findings	Single-cell isolation method	Data availability/ weblink	Reference
Wang *et al* (2021)	Human LT	10x Chromium	Immune Endothelia	Describe the heterogeneity of macrophages, ECs and lymphocytes during perioperative periods of LT.	*Ex vivo* enzymatic digestion	GEO GSE171539	19
Shao *et al* (2024)	Human LT	10x Chromium	Epithelia Immune Endothelia Mesenchyme	Identify a pathogenic immune niche (MAIT, *Gzmb*+*Gzmk*+ NK cells, *S100a12*+ neutrophils) associated with early allograft dysfunction post-LT.	GentleMACS Tissue Dissociator (Miltenyi Biotec)	GEO GSE189539	20
Yang *et al* (2021)	Rat Steatotic LT	BD Rhapsody	Epithelia Immune	Identify $Csf3^{high}$ KCs and $Xcr1^{high}$ DCs mediating severer steatotic graft injury after LT in rats.	*Ex vivo* enzymatic digestion	Genome Sequence Archive CRA004061	21
Other injury models							
Kolodziejczyk *et al* (2020)	Mouse TAA (injury)	10x Chromium	Immune Endothelia Mesenchyme	Identify activated HSCs, ECs and KCs under MYC-dependent programme in driving ALI.	*In vivo* enzymatic perfusion, NPC merged with sorted HSCs	ArrayExpress E-MTAB-8263	9
Zhang *et al* (2023)	Mouse Septic (injury)	10x Chromium	Epithelia Immune Endothelia Mesenchyme	Describe the heterogeneity of hepatocytes, macrophages, neutrophils and ECs in sepsis-induced liver injury.	GentleMACS Tissue Dissociator (Miltenyi Biotec)	Genome Sequence Archive CRA010041	22
Fischer *et al* (2022)	Mouse Electroporation (repair)	Drop-seq	Immune	Elucidate that neutrophils do not synthesise matrix but carry pre-existing matrix to initiate liver repair in an integrin-dependent manner.	*Ex vivo* enzymatic digestion	GEO GSE198828	23
Posthepatectomy regeneration							
Walesky *et al* (2020)	Mouse PHx	Seq-Well	Epithelia	Elucidate transcriptional shifts in hepatocytes from different zones during liver repair.	*In vivo* enzymatic perfusion	GEO GSE136679	10
Chen *et al* (2020)	Mouse PHx	10x Chromium	Epithelia	Describe dynamics of zonal hepatocytes during liver regeneration post-PHx.	*In vivo* enzymatic perfusion, hepatocytes captured	GEO GSE158866 GEO GSE158873	24
Xu *et al* (2024)	Mouse PHx	MGI DNBelab C Series HT Stereo-seq	Epithelia Immune Endothelia Mesenchyme	Describe spatiotemporal dynamics of hepatocytes, HSCs and ECs during liver regeneration; identify *Tbl1xr1* in hepatocytes as a rheostat for promoting regeneration.	*In vivo* enzymatic perfusion	CNGBdb CNP0002310 https://db.cngb.org/stomics/lista/	25

ALF, acute liver failure; ALI, acute liver injury; APAP, acetaminophen; CCl_4, carbon tetrachloride; CITE-seq, cellular indexing of transcriptomes and epitopes by sequencing; CNGBdb, China National GeneBank DataBase; DC, dendritic cell; EC, endothelial cell; GEO, Gene Expression Omnibus; HSC, hepatic stellate cell; HT, high-throughput; KC, Kupffer cell; LT, liver transplantation; MAIT, mucosal-associated invariant T cell; MP, mononuclear phagocyte; MYC, MYC proto-oncogene, bHLH transcription factor; N/A, not available; NAE, non-A-E hepatitis; NK, natural killer; NPC, non-parenchymal cell; PHx, partial hepatectomy; sn, single nucleus; Stereo-seq, spatial enhanced resolution omics-sequencing; TAA, thioacetamide.

Table 2 Comparison between single-cell RNA sequencing and single-nucleus RNA sequencing in liver research

	Single-cell RNA sequencing	Single-nucleus RNA sequencing
Samples	Single cells from fresh tissues	Nuclei from single-cell suspensions or frozen tissues
Major cell types recovered	Most of major cell types: hepatocytes, cholangiocytes, mesenchymal cells, endothelial cells, macrophages, neutrophils, lymphocytes	Hepatocytes, cholangiocytes, mesenchymal cells, endothelial cells, Kupffer cells, resident NK cells
Advantages	► Can enrich specific cell types based on research focus. ► Preferred for studies involving immune response. ► Provide a more comprehensive view of the transcriptome, including both nuclear and cytoplasmic transcripts.	► Flexible workflow, immediate processing not required. ► Ideal for small or easily degradable specimens. ► Identify rare cell types. ► Capture nascent transcripts.
Disadvantages	► Require immediate processing of liver tissues. ► Cell isolation may cause the loss of rare or vulnerable cells. ► Using microfluidic chips may fail to encapsulate and profile large cells like hepatocytes due to the physical limitations of the microfluidic chips.	► Limitations in investigating immune cells. ► Inability to sequence cytoplasmic RNA.

NK, natural killer.

endothelial cells, lipid-associated macrophages and liver-resident immune cells (including Kupffer cells (KCs) and tissue-resident natural killer cells) from patients with decompensated cirrhosis.[26] SnRNA-seq is favourable in identifying rare cells or rare subtypes of cells as it avoids single-cell dissociation, which can lead to the loss of rare cells.[29] Conversely, scRNA-seq is preferable when immune cells are of interest, as it can recover nearly all major cell types.[30] Notably, a signature of upregulation of stress-related genes was observed in scRNA-seq data compared with snRNA-seq data, probably due to cell dissociation-induced stress in scRNA-seq.[26] Overall, snRNA-seq can either complement scRNA-seq or serve as a viable alternative when it is challenging to obtain fresh and sufficient samples, such as the liver biopsies from patients. As summarised in table 1, only one study primarily employed snRNA-seq to elucidate the heterogeneity of hepatocytes during liver repair,[11] while scRNA-seq was performed in all other studies, indicating that scRNA-seq remains the preferred method for research on ALI and regeneration.

The scRNA-seq methods differ in how cell-of-origin transcripts are tagged and how sequencing libraries are generated. This review will not cover low-throughput, plate-based methods like Smart-seq2[31] and CEL-Seq2,[32] which have been thoroughly discussed in previous reviews.[5 33] ScRNA-seq methods use either a droplet-based microfluidic system or a microwell/platewell-based technology (table 3) to compartmentalise single cells or nuclei with microbeads. In droplet-based microfluidic systems, such as 10x Chromium,[34] Drop-seq,[35] MGI DNBelab C Series HT and inDrops,[36] cells are encapsulated within droplets and captured. The other platforms such as Seq-Well,[37] BD Rhapsody,[38] Singleron SCOPE-chip, sci-RNA-seq[39] and automated high-throughput Smart-seq3[40] rely on gravity to settle cells into wells. A comparative analysis of these methods suggests that inDrops exhibits low sensitivity, while sci-RNA-seq yields a relatively low fraction of exonic reads (~29%). Additionally, Seq-Well and Drop-seq identify fewer genes per cell compared with 10x Chromium.[33] The 10x Chromium, MGI DNBelab C

Table 3 Comparison between droplet-based microfluidic system and microwell/platewell-based technology in single-cell RNA sequencing

	Droplet-based microfluidic system	Microwell/platewell-based technology
Methods	10x Chromium Drop-seq MGI DNBelab C Series HT inDrops	Seq-Well BD Rhapsody Singleron SCOPE-chip sci-RNA-seq Automated high-throughput Smart-seq3
Capture approach	Droplet-based	Gravity-based
Shear stress	High	Low

HT, high-throughput.

Series HT, BD Rhapsody and Singleron SCOPE-chip are currently widely used for scRNA-seq research and have been successfully commercialised. Notably, gravity-based cell capture can reduce shear stress, enabling the investigation of vulnerable cells like eosinophils at the single-cell level[41] and opening new research opportunities.

Sequencing-based ST methods can be categorised based on spatial indexing strategies. These include microarray approaches such as probe/polyA-based 10x Visium,[42] Decoder-seq[43] and Seq-Scope[44]; bead-based methods including HDST,[45] BMKMANU ST, Slide-seqV2[46] and Slide-tag[47]; polony gel/nanowell-based technologies such as PIXEL-seq,[48] Stereo-seq[49] and Salus-STS[50]; and microfluidic systems such as DBiT-seq.[51] You and colleagues[52] developed a standardised benchmarking pipeline to ensure a fair and consistent comparison of various aforementioned ST methods except Seq-Scope. Among these ST methods, probe-based 10x Visium, Slide-tag and Stereo-seq demonstrated the highest read-capturing efficiency in selected regions. However, Slide-tag could not undergo sensitivity evaluation due to the inability to generate binary alignment map (BAM) files and perform subsequent downsampling.[52] Among these methods, 10x Visium remains the most widely used ST method. However, its resolution is relatively low, with a centre-to-centre distance of 100 µm between spots.[52] In contrast, Stereo-seq offers a significantly higher resolution, with a centre-to-centre distance of just 500 nm,[49] allowing precise immune cell analysis at the single-cell level. The resolution limitation of 10x Visium later was addressed with the introduction of 10x Visium HD in 2024, which features 2 µm×2 µm barcoded squares,[53] making it as practical as Stereo-seq for diverse ST studies.

FROM LIVER TISSUES TO SINGLE CELLS

The initial step of scRNA-seq experiments (figure 1) involves liver tissue dissociation into single-cell suspensions through either *in vivo* enzymatic perfusion or *ex vivo* digestion with collagenase. In rodents, the classic *in vivo* two-step collagenase perfusion technique is widely used to isolate primary hepatocytes and non-parenchymal cells (NPCs) from healthy and diseased livers.[54 55] However, the *in vivo* enzymatic perfusion is not suitable for investigating focal lesions in the livers[23] or when using human biopsies.[19 20] In such cases, the *ex vivo* digestion approach is used. This method can be easily scaled up, enabling the isolation of liver cells from multiple liver samples at the same time. Unlike *in vivo* enzymatic perfusion that completely digests liver tissues, the *ex vivo* digestion method, even with GentleMACS Tissue Dissociator (Miltenyi Biotec), results in the loss of hepatocytes and only partially recovers some NPCs like HSCs.[8 19 20 56]

Hepatocytes constitute approximately 60% of the total human liver cells, and each produces nearly twice as many reads as each NPC in scRNA-seq data,[57] significantly impacting sequencing depth and subsequent analysis for NPCs. To ensure proper representation of the cells of interest and to enrich viable cells to meet scRNA-seq requirements, cell enrichment techniques, including fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS), are often used. FACS is remarkably efficient and versatile, whereas MACS is simpler, scalable and faster for preventing transcript

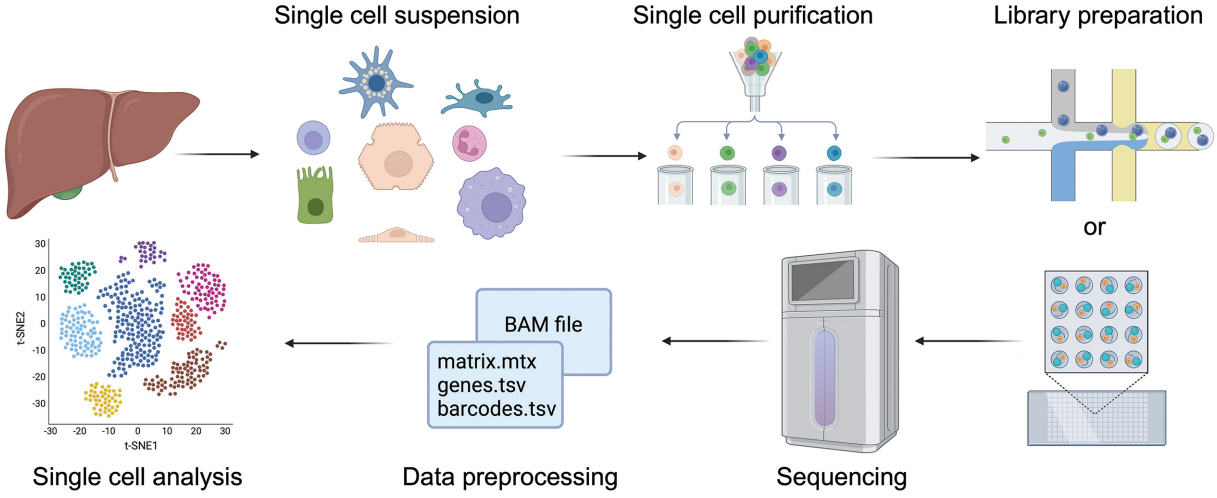

Figure 1 Workflow of single-cell RNA sequencing experiments. Initially, cells are dissociated from liver tissues into single-cell suspensions using *in vivo* enzymatic perfusion or *ex vivo* digestion methods. The cells of interest are then enriched from the suspensions through FACS or MACS. Cells are then processed using either high-throughput droplet-based microfluidic systems or microwell-based systems, followed by single-cell RNA lysis, reverse transcription, cDNA library construction and sequencing. The raw sequencing data are preprocessed through bioinformatic pipelines to generate count metrics, which are then used for computational analyses such as clustering, annotation, trajectory inference, regulatory network analysis or cell-cell communication analysis. Created with BioRender. BAM, binary alignment map; FACS, fluorescence-activated cell sorting; MACS, magnetic-activated cell sorting.

degradation, although it may not achieve the same purity levels as FACS.[58] However, the high-pressure nature of FACS can exert mechanical stress on cells during the sorting process, potentially affecting cellular viability and gene expression profiles. In contrast, MACS reduces the shear stress, better preserving fragile cell populations and preventing transcript alteration.[41 58]

Following sequencing (figure 1), the generated transcriptomic data undergo computational analysis using a variety of well-established R and Python-based tools for scRNA-seq (table 4) and ST (table 5). Cell annotation in single-cell transcriptomics, which involves identifying and labelling individual cells based on their gene expression profiles, is a crucial step for the accurate interpretation of scRNA-seq data. In recent years, both automatic cell annotation tools[59–61] and manual annotation have been applied for the analysis of single-cell transcriptomics[62] (table 4). With the advent of generative artificial intelligence (AI), deep learning-based cell annotation approaches, such as scDeepSort,[63] scBERT,[64] TOSICA,[65] scRGCL[66] and GPTCelltype,[67] have demonstrated high precision in distinguishing closely related cell types. However, rare cell types with extremely low representation in reference datasets remain challenging to be identified accurately. This limitation may be mitigated through fine-tuning on reference datasets.[68] In particular, the establishment of a comprehensive reference dataset for liver cells is urgently needed to enhance annotation accuracy in hepatic single-cell studies.

NOVEL INSIGHTS GAINED FROM SINGLE-CELL TRANSCRIPTOMIC STUDIES

Single-cell transcriptomics has significantly advanced our understanding of cellular heterogeneity in key cell types—such as hepatocytes, HSCs, endothelial cells and macrophages—during ALI and regeneration. In this section, we review the main findings from single-cell transcriptomic analysis of various liver cell types and discuss potential therapeutic targets aimed at mitigating injury and promoting tissue repair in acute liver diseases. Notably, many of these studies have focused on repair processes following pericentral injury induced by acetaminophen (APAP) overdose (table 1), which shares similar mechanisms in mice after carbon tetrachloride (CCl_4) insult. We also extend the discussion of the novel mechanisms underlying APAP-induced ALI to ischaemia/reperfusion (I/R) injury. The regenerative mechanisms are further revealed in mice post-partial hepatectomy (PHx).

Hepatocytes

ALI can be rapidly resolved by surviving hepatocytes without the involvement of progenitor cells.[69] In cases of hepatotoxin-induced liver injury, such as that caused by CCl_4 or APAP, midzonal (zone 2) and the remaining pericentral (zone 3) hepatocytes proliferate to repopulate necrotic pericentral areas following excessive hepatocyte loss.[16 70] Additionally, periportal (zone 1) hepatocytes exhibited increased expression of proliferation markers, such as *Mki67*, indicating their participation in compensatory hepatocyte proliferation after acute injury, although their proliferative activity is not as pronounced as that of pericentral hepatocytes.[12] Wang *et al*[16] further analysed the temporal changes in the transcriptomic landscape in hepatocytes during repair following CCl_4-induced liver injury. By comparing ST data from various time points postinjury with that acquired from undamaged control livers, they demonstrated significant increases in gene expressions associated with cell-cycle progression, proliferation-associated anabolism, fetal liver development and cell motility. Notably, the peak of proliferative activity occurred on day 2, fetal liver development pathways peaked between day 2 and day 3 and cell motility pathways peaked on day 3,[16] suggesting a dynamic progression of hepatocyte repopulation during liver repair.

Two single-cell transcriptomic studies reported a predominant accumulation of fetal-like (*Afp*$^+$*Cdh17*$^+$*Spp1*$^+$) hepatocytes and migratory (*Anxa2*$^+$) hepatocytes at the interface between necrotic areas and undamaged tissue within the pericentral zone during repair following APAP overdose.[11 12] The fetal-like hepatocytes exhibited a mesenchymal-like morphology with elongated extrusions extending into the damaged region.[12] Additionally, these cells showed upregulated pathways associated with ribosomal and proteasomal activities, potentially translating new pericentral proteins while simultaneously degrading existing periportal/mid-zonal proteins.[12]

The *Anxa2*$^+$ hepatocytes were initially identified as a subcluster of hepatocytes in the livers of patients with APAP-induced ALF and non-hepatitis A-E ALF but were absent in healthy livers. They were also observed in human chronic liver diseases caused by various aetiologies and different mouse models of liver injury.[11] Gene ontology analysis of the *Anxa2*$^+$ hepatocytes suggests a migratory cell phenotype. Immunohistological (immunohistochemistry) staining further confirmed their motile morphology, characterised by ruffled membranes and extending lamellipodia.[11] *Anxa2* in hepatocytes serves as a marker and directly promotes hepatocyte migration by inducing F-actin expression and reducing hepatocyte circularity. Knockdown of *Anxa2* in hepatocytes impaired their migration both *in vivo* and *in vitro*, leading to delayed wound closure in mouse livers following APAP exposure.[11] Ben-Moshe *et al*[12] found that *Afp* and *Cdh17*, markers of fetal-like hepatocytes, peaked in pericentral hepatocytes on day 2 post-APAP injection in mice, while *Anxa2* induction was observed on day 2 and became more pronounced by day 3, suggesting a transition from fetal-like to migratory hepatocytes. Cell-cell communication analysis attributed *Anxa2* induction in hepatocytes to HSC-derived transforming growth factor beta,[11] which has previously been reported to induce fetal-like epithelial reprogramming.[71] Although the precise mechanisms of *Axna2* induction have yet to be fully elucidated, these findings suggest that *Anxa2* may serve as a promising therapeutic target for promoting repair after ALI.

Table 4 Strategies of single-cell RNA sequencing analysis

Exemplar packages	Language	URL
Data preprocessing: from raw data to count or read matrix		
Cell Ranger	Shell	https://www.10xgenomics.com/support/software/cell-ranger/latest
CeleScope	Shell	https://github.com/singleron-RD/CeleScope
Other pipelines from companies with single-cell RNA sequencing service		
From count or read matrix to visualisation		
Seurat	R	https://satijalab.org/seurat/
Scanpy	Python	https://scanpy.readthedocs.io/en/stable/
SingleCellExperiment	R	https://bioconductor.org/packages/release/bioc/html/SingleCellExperiment.html
scvi-tools	Python	https://scvi-tools.org/
Doublet removal		
DoubletFinder	R	https://github.com/chris-mcginnis-ucsf/DoubletFinder
scDblFinder	R	https://github.com/plger/scDblFinder
DoubletDetection	Python	https://github.com/JonathanShor/DoubletDetection
Vaeda	Python	https://github.com/kostkalab/vaeda
Scrublet	Python	https://github.com/AllonKleinLab/scrublet
Batch effect correction		
Harmony	R	https://github.com/immunogenomics/harmony
Harmonypy	Python	https://github.com/slowkow/harmonypy
Scanorama	Python	https://github.com/brianhie/scanorama
Automatic cell annotation		
SingleR	R	https://github.com/dviraran/SingleR
CellTypist	R, Python	https://github.com/Teichlab/celltypist
Databases for manual annotation		
PanglaoDB	Online	https://panglaodb.se/
CellMarker 2.0	Online	http://www.bio-bigdata.center/
RNA velocity		
Velocyto	R, Python	https://velocyto.org/
scVelo	Python	https://scvelo.readthedocs.io/en/stable/
Dynamo	Python	https://github.com/aristoteleo/dynamo-release
Trajectory inference		
Monocle	R	https://cole-trapnell-lab.github.io/monocle3/
Slingshot	R	https://github.com/kstreet13/slingshot
pySlingshot	Python	https://github.com/mossjacob/pyslingshot
PAGA	Python	https://github.com/theislab/paga
Ligand-receptor interactions and cell-cell communication		
CellChat	R	https://github.com/jinworks/CellChat
NicheNet	R	https://github.com/saeyslab/nichenetr
CellphoneDB	Python	https://github.com/ventolab/CellphoneDB
Gene regulatory networks		
SCENIC	R	https://github.com/aertslab/SCENIC
pySCENIC	Python	https://github.com/aertslab/pySCENIC
scGRN	Online	https://bio.liclab.net/scGRN/index.php

Table 5 Strategies of spatial transcriptomic analysis

Exemplar packages	Language	URL
Data preprocessing		
Space Ranger	Shell	https://www.10xgenomics.com/support/software/space-ranger/latest
SAW	Shell	https://en.stomics.tech/service/new-saw-operation-manual.html
Secondary analysis, visualisation and integration		
Seurat	R	https://satijalab.org/seurat/articles/spatial_vignette.html
SPATA2	R	https://themilolab.github.io/SPATA2/
Semla	R	https://github.com/ludvigla/semla
Giotto	R, Python	https://github.com/RubD/Giotto
Squidpy	Python	https://squidpy.readthedocs.io/en/stable/index.html
stLearn	Python	https://github.com/BiomedicalMachineLearning/stLearn
scvi-tools	Python	https://scvi-tools.org/
SpaGCN	Python	https://github.com/jianhuupenn/SpaGCN

Hepatic stellate cells

HSCs, located in the space of Disse between hepatocytes and liver sinusoid endothelial cells (LSECs), comprise approximately 5–10% of the total liver cell population.[72] While HSCs have been implicated in contributing to ALI in various mouse models,[73 74] they play a crucial role in maintaining liver homeostasis by supporting hepatocyte proliferation[75] and promoting hepatectomy or injury-induced liver regeneration by producing growth factors and remodelling extracellular matrix (ECM).[72 76 77]

Kolodziejczyk *et al*[9] identified a cluster of activated HSCs, which emerged 20 hours after APAP overdose. These cells are characterised by a significant induction of *Acta2* expression while lacking *Col1a1* production, distinguishing them from the activated HSCs observed in fibrotic livers. These activated HSCs were predominantly localised in periportal and mid-lobular zones[12] and exhibited a contractile phenotype, driven by the high expression levels of stress fibre genes compared with quiescent and fibrotic HSCs. Additionally, they displayed an immunomodulatory profile, producing various cytokines and chemokines.[9] Notably, these activated HSCs may contribute to APAP-induced ALI, as pharmacological inhibition of MYC proto-oncogene, bHLH transcription factor (MYC), the key regulator of 'activated HSCs', was shown to mitigate liver injury in mice.[9]

Following APAP exposure, the expression of *Acta2* and production of cytokines by the activated HSCs rapidly decreased to basal levels by day 2. Concurrently, ECM-related genes, such as *Col1a1* and *Col1a2*, surged in pericentral and mid-zonal HSCs, suggesting a phenotypic shift from an injury-associated state to one facilitating liver repair.[12] Notably, periportal HSCs exhibited preferential proliferation, peaking on day 2, potentially serving as a reservoir of HSCs that migrate into damaged areas to support regeneration.[12] In contrast, during liver regeneration induced by PHx, HSCs across all zones initially repressed ECM-related genes by day 1, followed by increased ECM remodelling activity peaking on day 3. HSCs have also been reported as the key source of hepatocyte growth factor (HGF),[78] a critical regulator of liver regeneration post-PHx.[79] The spatiotemporal study has shown that HGF expression begins in mid-zonal HSCs as early as 8 hours post-PHx, peaks by day 1 and remains elevated on day 2 across all HSCs, confirming the crucial role of HSCs in promoting liver regeneration through HGF signalling.[25] Collectively, these findings further underscore the diverse roles of HSCs in ALI and regeneration, highlighting the need for developing strategies to modify HSCs with injury-preventing and repair-promoting functions.

Endothelial cells

LSECs are integral to liver function, playing a pivotal role in both ALI and regeneration as reviewed previously.[80 81] They are the predominant sources of WNT ligands, such as WNT2 and WNT9b, which are crucial for maintaining liver zonation and facilitating repair processes.[81 82] Recent ST studies have provided insights into the spatiotemporal changes of proreparative LSECs in mouse liver repair, revealing that these cells exhibit zonation patterns as hepatocytes and HSCs. In normal livers, *Wnt2* is predominantly expressed by pericentral LSECs, while *Wnt9b* is almost exclusively expressed by pericentral liver vascular endothelial cells (LVECs).[12] However, following APAP exposure, *Wnt2* expression increases in pericentral LVECs, but not in pericentral LSECs, reaching a peak at approximately twice the basal level on day 2. Conversely, the increase in *Wnt9b* levels occurs only in pericentral LSECs, not in pericentral LVECs.[12] In the mouse model of PHx, the upregulation of *Wnt2* and *Wnt9b* begins as early as 8 hours and peaks on day 1 in pericentral LSECs.[25] Cell-cell communication analysis further indicates that enhanced interaction of macrophage migratory

inhibition factor or *Cxcl12* (encoding SDF-1) with *Ackr3* (encoding CXCR7) in pericentral LSECs following PHx might account for the upregulation of WNTs.[25] Notably, shear stress, along with *Hbegf* induction, is observed in all LSECs at 8 hours after PHx.[25] This aligns with the study demonstrating that PHx-induced changes in blood flow per unit mass lead to mechanical stretch and shear stress on LSECs, subsequently triggering heparin-binding EGF-like growth factor (HB-EGF) production through YAP1 activation.[83] Together, single-cell transcriptomic studies have deepened our understanding of the key mechanisms underlying endothelial cell-mediated liver repair.

Macrophages

Liver macrophages, consisting of resident macrophages, ie, KCs and monocyte-derived macrophages (MoMFs), play important roles in liver injury and repair. Following acute insults, KCs release proinflammatory cytokines and chemokines that contribute to liver injury, and they subsequently undergo death to initiate resolution.[84] Hepatic macrophages are eventually replenished during the tissue repair phase.[85] In response to chemokines such as CCL2, *Ly6C*^{high} MoMFs infiltrate damaged livers, exacerbating liver inflammation, and then transition to *Ly6C*^{low} MoMFs, which exhibit anti-inflammatory and restorative functions by expressing matrix metalloproteinases, growth factors and phagocytosis-related genes.[84] Single-cell transcriptomic studies have advanced our understanding of the heterogeneity of KCs and MoMFs in the context of liver injury and repair.[86]

On acute insult, only a subset of KCs become activated.[9 12 19] Li *et al*[8] identified a cluster of *Cxcl2*$^{+}$ KCs, whose number increased as early as 3 hours following APAP exposure. These KCs are involved in proinflammatory responses, reactive oxygen species production and neutrophil recruitment.[8] An important discovery in liver macrophages is the identification of *Trem2*$^{+}$ MoMFs, which are also observed in various human diseases and mouse models.[87] *Trem2*-deficient mice exhibited attenuated I/R–induced injury,[88] yet augmented liver injury induced by APAP and CCl_4,[89 90] suggesting the role of *Trem2* in injury processes varies depending on the context of ALI. The underlying mechanisms of *Trem2* and *Trem2*$^{+}$ macrophages in ALI remain unknown and require further investigation.

Trem2 expression in MoMFs and the number of *Trem2*$^{+}$ MoMFs increase significantly during liver repair after acute injury, peaking on day 3 after APAP treatment.[12 15] *Trem2*$^{+}$ MoMFs, whose gene profiles largely correlate with previously reported *Ly6C*^{low} restorative MoMFs and recovered KCs, show pathways related to phagocytosis and cell clearance. Trajectory analysis shows that they may originate from *Ly6C*^{low} MoMFs.[13 85] *Trem2* deletion in macrophages impedes dying cell clearance, thereby impairing liver repair in mice with both drug and I/R-induced liver injury.[15 88] Notably, a cluster of *Trem2*$^{+}$ KCs emerge during the early repair phase following APAP exposure with much lower *Trem2* transcript levels than MoMF.[8 12 15] These *Trem2*$^{+}$ KCs originate from resident KCs instead of bone marrow and exhibit a distinct gene signature compared with *Trem2*$^{+}$ MoMFs. Although *Trem2*$^{+}$ KCs also possess the ability for phagocytosis, *Trem2* deletion exclusively in KCs fails to affect liver repair following acute insult.[15]

KCs repopulate gradually during liver repair processes. Li *et al*[18] conducted scRNA-seq experiments to investigate KCs on day 28 after CCl_4 injection and revealed that monocyte-derived KCs (moKCs) replenished embryonic KCs and dominated the KC pools. The gene profiles of moKCs are not identical to those of embryonic KCs. For instance, moKCs exhibited stronger efferocytosis than embryonic KCs in *ex vivo* experiments. Notably, *Trem2*$^{+}$ moKCs greatly expand, and *Mmp12*$^{+}$ moKCs emerge in the KC pool in the recovered liver. Both are reparative with a wound healing profile, but *Trem2*$^{+}$ moKCs are associated with phagocytosis and apoptotic cell clearance, while *Mmp12*$^{+}$ moKCs are related to the regulation of endocytosis and ECM organisation.[18] Single-cell transcriptomic studies have significantly advanced our comprehension of macrophage heterogeneity within livers, which is crucial for the development of macrophage-based therapies.

CONCLUSION AND PERSPECTIVES

Single-cell transcriptomic technologies have enhanced our understanding of ALI and liver regeneration by providing detailed insights into cellular heterogeneity, dynamic changes and cell-cell crosstalk. These approaches have uncovered previously unrecognised cell states and molecular mechanisms, particularly in key cell populations such as hepatocytes, HSCs, endothelial cells and macrophages (figure 2), thereby contributing to our knowledge of the underlying mechanisms of liver injury and repair. Despite these advancements, several challenges and opportunities remain. First, while significant progress has been made in understanding certain cell types, the roles of others, such as granulocytes and lymphocytes, in ALI and repair are not fully understood and require further investigation at the single-cell level. Second, the diversity in cell isolation methods and single-cell platforms complicates the integration of data from different laboratories, potentially leading to data resource wastage and hindering new discoveries. Standardisation of single-cell protocols from tissue processing to sequencing and analysis pipelines is essential to address this issue. Third, transcriptomic data from scRNA-seq and ST may not fully capture the complexities of the injury and repair processes of the liver. The development of single-cell omics, such as single-cell proteomics and single-cell metabolomics, is necessary to provide a more comprehensive understanding. Fourth, integrating AI into single-cell analysis holds significant potential to deepen our findings. As single-cell technologies continue to evolve and

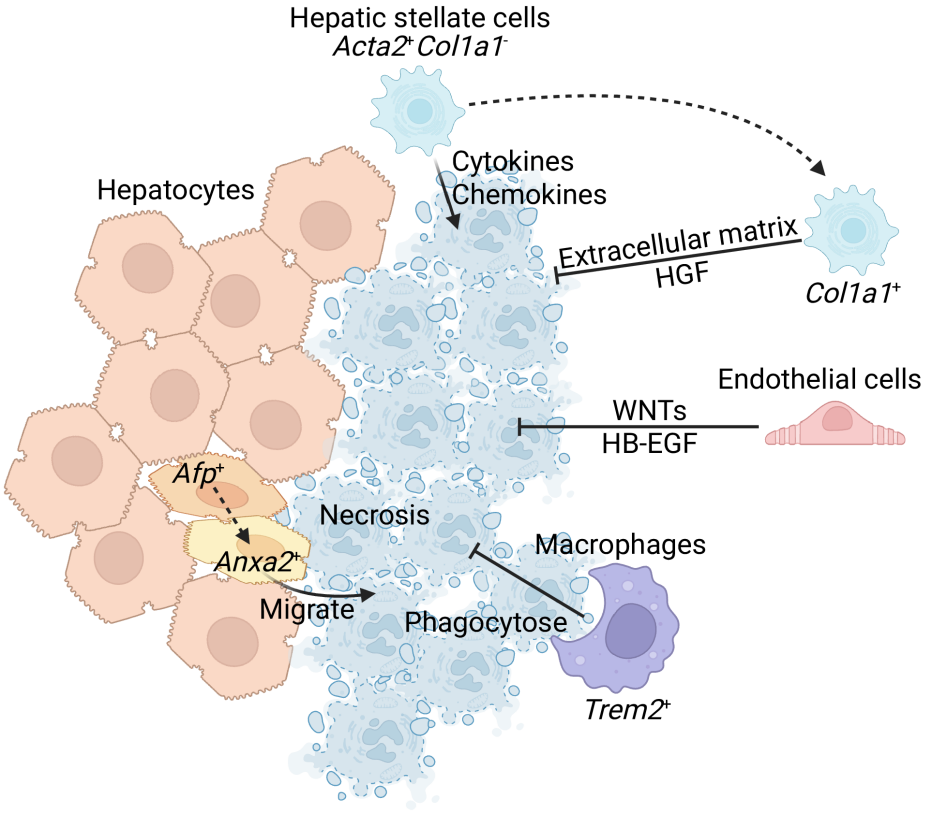

Figure 2 Novel insights gained from single-cell transcriptomic studies. In single-cell transcriptomic studies, fetal-like (*Afp*$^+$*Cdh17*$^+$*Spp1*$^+$) hepatocytes and migratory (*Anxa2*$^+$) hepatocytes predominantly accumulate at the interface between necrotic areas and undamaged tissue, facilitating wound closure following ALI. Activated HSCs following ALI display distinct functions during different stages: *Acta2*$^+$*Col1a1*$^-$ HSCs secrete cytokines and chemokines to drive inflammation during the injury phase, whereas *Col1a1*$^+$ HSCs are involved in HGF production and extracellular matrix remodelling during the repair phase. Pericentral endothelial cells serve as a primary source of WNT2 and WNT9B, which are crucial for liver homeostasis and repair, while endothelial cells across all zones produce HB-EGF in response to shear stress, promoting liver regeneration. Additionally, *Trem2*$^+$ macrophages play a vital role in resolving injury by phagocytosing dead cells. Collectively, these cell types coordinate to orchestrate effective liver repair post-ALI. Created with BioRender. ALI, acute liver injury; HB-EGF, heparin-binding EGF-like growth factor; HGF, hepatocyte growth factor; HSCs, hepatic stellate cells.

become more accessible, their application in liver research is expected to yield additional insights into the cellular and molecular mechanisms underlying ALI and regeneration. These advances may lead to the development of more effective therapeutic strategies for patients with acute liver diseases.

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REFERENCES

1 Stravitz RT, Lee WM. Acute liver failure. *Lancet* 2019;394:869–81.
2 Tujios S, Stravitz RT, Lee WM. Management of Acute Liver Failure: Update 2022. *Semin Liver Dis* 2022;42:362–78.
3 Liu Q, Wang S, Fu J, *et al*. Liver regeneration after injury: Mechanisms, cellular interactions and therapeutic innovations. *Clin Transl Med* 2024;14:e1812.
4 Lin P, Yan X, Jing S, *et al*. Single-cell and spatially resolved transcriptomics for liver biology. *Hepatology* 2024;80:698–720.
5 Saviano A, Henderson NC, Baumert TF. Single-cell genomics and spatial transcriptomics: Discovery of novel cell states and cellular interactions in liver physiology and disease biology. *J Hepatol* 2020;73:1219–30.

6 Ramachandran P, Matchett KP, Dobie R, *et al*. Single-cell technologies in hepatology: new insights into liver biology and disease pathogenesis. *Nat Rev Gastroenterol Hepatol* 2020;17:457–72.
7 Umbaugh DS, Ramachandran A, Jaeschke H. Spatial Reconstruction of the Early Hepatic Transcriptomic Landscape After an Acetaminophen Overdose Using Single-Cell RNA-Sequencing. *Toxicol Sci* 2021;182:327–45.
8 Li X, Zhi Y, Li J, *et al*. Single-cell RNA sequencing to reveal non-parenchymal cell heterogeneity and immune network of acetaminophen-induced liver injury in mice. *Arch Toxicol* 2023;97:1979–95.
9 Kolodziejczyk AA, Federici S, Zmora N, *et al*. Acute liver failure is regulated by MYC- and microbiome-dependent programs. *Nat Med* 2020;26:1899–911.
10 Walesky CM, Kolb KE, Winston CL, *et al*. Functional compensation precedes recovery of tissue mass following acute liver injury. *Nat Commun* 2020;11:5785.
11 Matchett KP, Wilson-Kanamori JR, Portman JR, *et al*. Multimodal decoding of human liver regeneration. *Nature* 2024;630:158–65.
12 Ben-Moshe S, Veg T, Manco R, *et al*. The spatiotemporal program of zonal liver regeneration following acute injury. *Cell Stem Cell* 2022;29:973–89.
13 Song Q, Datta S, Liang X, *et al*. Type I interferon signaling facilitates resolution of acute liver injury by priming macrophage polarization. *Cell Mol Immunol* 2023;20:143–57.
14 Umbaugh DS, Nguyen NT, Smith SH, *et al*. The p21^{+} perinecrotic hepatocytes produce the chemokine CXCL14 after a severe acetaminophen overdose promoting hepatocyte injury and delaying regeneration. *Toxicology* 2024;504:153804.
15 De Ponti FF, Bujko A, Liu Z, *et al*. Spatially restricted and ontogenically distinct hepatic macrophages are required for tissue repair. *Immunity* 2025;58:362–80.
16 Wang S, Wang X, Shan Y, *et al*. Region-specific cellular and molecular basis of liver regeneration after acute pericentral injury. *Cell Stem Cell* 2024;31:341–58.
17 Zhao L, Jin Y, Donahue K, *et al*. Tissue Repair in the Mouse Liver Following Acute Carbon Tetrachloride Depends on Injury-Induced Wnt/β-Catenin Signaling. *Hepatology* 2019;69:2623–35.
18 Li W, Yang Y, Yang L, *et al*. Monocyte-derived Kupffer cells dominate in the Kupffer cell pool during liver injury. *Cell Rep* 2023;42:113164.
19 Wang L, Li J, He S, *et al*. Resolving the graft ischemia-reperfusion injury during liver transplantation at the single cell resolution. *Cell Death Dis* 2021;12:589.
20 Shao X, Wang Z, Wang K, *et al*. A Single-Cell Landscape of Human Liver Transplantation Reveals a Pathogenic Immune Niche Associated with Early Allograft Dysfunction. *Engineering* 2024;36:193–208.
21 Yang X, Lu D, Wang R, *et al*. Single-cell profiling reveals distinct immune phenotypes that contribute to ischaemia-reperfusion injury after steatotic liver transplantation. *Cell Prolif* 2021;54:e13116.
22 Zhang Q, Liu J, Shen J, *et al*. Single-cell RNA sequencing reveals the effects of capsaicin in the treatment of sepsis-induced liver injury. *MedComm (2020)* 2023;4:e395.
23 Fischer A, Wannemacher J, Christ S, *et al*. Neutrophils direct preexisting matrix to initiate repair in damaged tissues. *Nat Immunol* 2022;23:518–31.
24 Chen T, Oh S, Gregory S, *et al*. Single-cell omics analysis reveals functional diversification of hepatocytes during liver regeneration. *JCI Insight* 2020;5:e141024.
25 Xu J, Guo P, Hao S, *et al*. A spatiotemporal atlas of mouse liver homeostasis and regeneration. *Nat Genet* 2024;56:953–69.
26 Van Melkebeke L, Verbeek J, Bihary D, *et al*. Comparison of the single-cell and single-nucleus hepatic myeloid landscape within decompensated cirrhosis patients. *Front Immunol* 2024;15:1346520.
27 Habib N, Avraham-Davidi I, Basu A, *et al*. Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat Methods* 2017;14:955–8.
28 Habib N, Li Y, Heidenreich M, *et al*. Div-Seq: Single-nucleus RNA-Seq reveals dynamics of rare adult newborn neurons. *Science* 2016;353:925–8.
29 Andrews TS, Atif J, Liu JC, *et al*. Single-Cell, Single-Nucleus, and Spatial RNA Sequencing of the Human Liver Identifies Cholangiocyte and Mesenchymal Heterogeneity. *Hepatol Commun* 2022;6:821–40.
30 Wen F, Tang X, Xu L, *et al*. Comparison of single-nucleus and single-cell transcriptomes in hepatocellular carcinoma tissue. *Mol Med Rep* 2022;26:339.
31 Picelli S, Björklund ÅK, Faridani OR, *et al*. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nat Methods* 2013;10:1096–8.
32 Hashimshony T, Senderovich N, Avital G, *et al*. CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. *Genome Biol* 2016;17:77.
33 Ding J, Adiconis X, Simmons SK, *et al*. Systematic comparison of single-cell and single-nucleus RNA-sequencing methods. *Nat Biotechnol* 2020;38:737–46.
34 Zheng GXY, Terry JM, Belgrader P, *et al*. Massively parallel digital transcriptional profiling of single cells. *Nat Commun* 2017;8:14049.
35 Macosko EZ, Basu A, Satija R, *et al*. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. *Cell* 2015;161:1202–14.
36 Klein AM, Mazutis L, Akartuna I, *et al*. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* 2015;161:1187–201.
37 Gierahn TM, Wadsworth MH 2nd, Hughes TK, *et al*. Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput. *Nat Methods* 2017;14:395–8.
38 Yuan J, Sims PA. An Automated Microwell Platform for Large-Scale Single Cell RNA-Seq. *Sci Rep* 2016;6:33883.
39 Cao J, Packer JS, Ramani V, *et al*. Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science* 2017;357:661–7.
40 Chuang H-C, Li R, Huang H, *et al*. Single-cell sequencing of full-length transcripts and T-cell receptors with automated high-throughput Smart-seq3. *BMC Genomics* 2024;25:1127.
41 Borrelli C, Gurtner A, Arnold IC, *et al*. Stress-free single-cell transcriptomic profiling and functional genomics of murine eosinophils. *Nat Protoc* 2024;19:1679–709.
42 Ståhl PL, Salmén F, Vickovic S, *et al*. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* 2016;353:78–82.
43 Cao J, Zheng Z, Sun D, *et al*. Decoder-seq enhances mRNA capture efficiency in spatial RNA sequencing. *Nat Biotechnol* 2024;42:1735–46.
44 Cho CS, Xi J, Si Y, *et al*. Microscopic examination of spatial transcriptome using Seq-Scope. *Cell* 2021;184:3559–72.
45 Vickovic S, Eraslan G, Salmén F, *et al*. High-definition spatial transcriptomics for in situ tissue profiling. *Nat Methods* 2019;16:987–90.
46 Stickels RR, Murray E, Kumar P, *et al*. Highly sensitive spatial transcriptomics at near-cellular resolution with Slide-seqV2. *Nat Biotechnol* 2021;39:313–9.
47 Russell AJC, Weir JA, Nadaf NM, *et al*. Slide-tags enables single-nucleus barcoding for multimodal spatial genomics. *Nature* 2024;625:101–9.
48 Fu X, Sun L, Dong R, *et al*. Polony gels enable amplifiable DNA stamping and spatial transcriptomics of chronic pain. *Cell* 2022;185:4621–33.
49 Chen A, Liao S, Cheng M, *et al*. Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell* 2022;185:1777–92.
50 Liao R, Fu D, Xu Z, *et al*. Sequencing-based spatial transcriptomics with scrna-seq sensitivity. *bioRxiv* [Preprint] 2025.
51 Liu Y, Yang M, Deng Y, *et al*. High-Spatial-Resolution Multi-Omics Sequencing via Deterministic Barcoding in Tissue. *Cell* 2020;183:1665–81.
52 You Y, Fu Y, Li L, *et al*. Systematic comparison of sequencing-based spatial transcriptomic methods. *Nat Methods* 2024;21:1743–54.
53 Guo CL, Wang CS, Wang ZC, *et al*. Granzyme K+CD8+ T cells interact with fibroblasts to promote neutrophilic inflammation in nasal polyps. *Nat Commun* 2024;15:10413.
54 Mederacke I, Dapito DH, Affò S, *et al*. High-yield and high-purity isolation of hepatic stellate cells from normal and fibrotic mouse livers. *Nat Protoc* 2015;10:305–15.
55 Charni-Natan M, Goldstein I. Protocol for Primary Mouse Hepatocyte Isolation. *STAR Protoc* 2020;1:100086.
56 Andreata F, Blériot C, Di Lucia P, *et al*. Isolation of mouse Kupffer cells for phenotypic and functional studies. *STAR Protoc* 2021;2:100831.
57 Su Q, Kim SY, Adewale F, *et al*. Single-cell RNA transcriptome landscape of hepatocytes and non-parenchymal cells in healthy and NAFLD mouse liver. *iScience* 2021;24:103233.
58 Meskauskaite Urben B, Krause T, Takizawa M, *et al*. Comparison of Live Cell Enrichment Methods for Single-Cell Rnaseq. *Blood* 2023;142:7179.
59 Huang Q, Liu Y, Du Y, *et al*. Evaluation of Cell Type Annotation R Packages on Single-cell RNA-seq Data. *Genomics Proteomics Bioinformatics* 2021;19:267–81.
60 Domínguez Conde C, Xu C, Jarvis LB, *et al*. Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science* 2022;376:eabl5197.
61 Pasquini G, Rojo Arias JE, Schäfer P, *et al*. Automated methods for cell type annotation on scRNA-seq data. *Comput Struct Biotechnol J* 2021;19:961–9.

62 Clarke ZA, Andrews TS, Atif J, *et al*. Tutorial: guidelines for annotating single-cell transcriptomic maps using automated and manual methods. *Nat Protoc* 2021;16:2749–64.
63 Shao X, Yang H, Zhuang X, *et al*. scDeepSort: a pre-trained cell-type annotation method for single-cell transcriptomics using deep learning with a weighted graph neural network. *Nucleic Acids Res* 2021;49:e122.
64 Yang F, Wang W, Wang F, *et al*. scBERT as a large-scale pretrained deep language model for cell type annotation of single-cell RNA-seq data. *Nat Mach Intell* 2022;4:852–66.
65 Chen J, Xu H, Tao W, *et al*. Transformer for one stop interpretable cell type annotation. *Nat Commun* 2023;14:223.
66 Yuan L, Sun S, Jiang Y, *et al*. scRGCL: a cell type annotation method for single-cell RNA-seq data using residual graph convolutional neural network with contrastive learning. *Brief Bioinform* 2024;26:bbae662.
67 Hou W, Ji Z. Assessing GPT-4 for cell type annotation in single-cell RNA-seq analysis. *Nat Methods* 2024;21:1462–5.
68 Cui H, Wang C, Maan H, *et al*. scGPT: toward building a foundation model for single-cell multi-omics using generative AI. *Nat Methods* 2024;21:1470–80.
69 Font-Burgada J, Shalapour S, Ramaswamy S, *et al*. Hybrid Periportal Hepatocytes Regenerate the Injured Liver without Giving Rise to Cancer. *Cell* 2015;162:766–79.
70 Lin Y-H, Wei Y, Zeng Q, *et al*. IGFBP2 expressing midlobular hepatocytes preferentially contribute to liver homeostasis and regeneration. *Cell Stem Cell* 2023;30:665–76.
71 Chen L, Qiu X, Dupre A, *et al*. TGFB1 induces fetal reprogramming and enhances intestinal regeneration. *Cell Stem Cell* 2023;30:1520–37.
72 Kamm DR, McCommis KS. Hepatic stellate cells in physiology and pathology. *J Physiol* 2022;600:1825–37.
73 Rani R, Gandhi CR. Stellate cell in hepatic inflammation and acute injury. *J Cell Physiol* 2023;238:1226–36.
74 Stewart RK, Dangi A, Huang C, *et al*. A novel mouse model of depletion of stellate cells clarifies their role in ischemia/reperfusion- and endotoxin-induced acute liver injury. *J Hepatol* 2014;60:298–305.
75 Trinh VQ-H, Lee T-F, Lemoinne S, *et al*. Hepatic stellate cells maintain liver homeostasis through paracrine neurotrophin-3 signaling that induces hepatocyte proliferation. *Sci Signal* 2023;16:eadf6696.
76 Yin C, Evason KJ, Asahina K, *et al*. Hepatic stellate cells in liver development, regeneration, and cancer. *J Clin Invest* 2013;123:66369:1902–10:.
77 Kitto LJ, Henderson NC. Hepatic Stellate Cell Regulation of Liver Regeneration and Repair. *Hepatol Commun* 2021;5:358–70.
78 Passino MA, Adams RA, Sikorski SL, *et al*. Regulation of hepatic stellate cell differentiation by the neurotrophin receptor p75NTR. *Science* 2007;315:1853–6.
79 Borowiak M, Garratt AN, Wüstefeld T, *et al*. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci U S A* 2004;101:10608–13.
80 Gracia-Sancho J, Caparrós E, Fernández-Iglesias A, *et al*. Role of liver sinusoidal endothelial cells in liver diseases. *Nat Rev Gastroenterol Hepatol* 2021;18:411–31.
81 McConnell MJ, Kostallari E, Ibrahim SH, *et al*. The evolving role of liver sinusoidal endothelial cells in liver health and disease. *Hepatology* 2023;78:649–69.
82 Hu S, Liu S, Bian Y, *et al*. Single-cell spatial transcriptomics reveals a dynamic control of metabolic zonation and liver regeneration by endothelial cell Wnt2 and Wnt9b. *Cell Rep Med* 2022;3:100754.
83 Wu Y, Li L, Li W, *et al*. Stretch-induced hepatic endothelial mechanocrine promotes hepatocyte proliferation. *Hepatology* 2024.
84 Wen Y, Lambrecht J, Ju C, *et al*. Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. *Cell Mol Immunol* 2021;18:45–56.
85 Zigmond E, Samia-Grinberg S, Pasmanik-Chor M, *et al*. Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. *J Immunol* 2014;193:344–53.
86 Guilliams M, Scott CL. Liver macrophages in health and disease. *Immunity* 2022;55:1515–29.
87 Colonna M. The biology of TREM receptors. *Nat Rev Immunol* 2023;23:580–94.
88 Han S, Li X, Xia N, *et al*. Myeloid Trem2 Dynamically Regulates the Induction and Resolution of Hepatic Ischemia-Reperfusion Injury Inflammation. *Int J Mol Sci* 2023;24:6348.
89 Perugorria MJ, Esparza-Baquer A, Oakley F, *et al*. Non-parenchymal TREM-2 protects the liver from immune-mediated hepatocellular damage. *Gut* 2019;68:533–46.
90 Chao S, Shan S, Liu Z, *et al*. Both TREM2-dependent macrophages and Kupffer cells play a protective role in APAP-induced acute liver injury. *Int Immunopharmacol* 2024;141:112926.